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Metabolism and sites of action of vitamin D in the kidney

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Before it exerts its biological action on its target tissues, the circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D), must be metabolized primarily by the kidney either to 1α,25-dihydroxyvitamin D (1,25$(OH)_2$D) or 24,25-hydroxyvitamin D (24,25$(OH)_2$D) by the enzymes 25(OH)D-1α-hydroxylase (1-hydroxylase) or 25(OH)D-24-hydroxylase (24-hydroxylase), respectively. Activities of these two enzymes are under tight hormonal and ionic control and there is, in general, a reciprocal change in activities of the two enzymes: When the 1-hydroxylase activity is stimulated, the 24-hydroxylase activity is suppressed and vice versa [1–3]. In this article we briefly review recent advances in the unique hormonal regulation of 1-hydroxylase and 24-hydroxylase and present the concept of two distinct 1-hydroxylase systems in the mammalian kidney. It has been appreciated that the kidney is among the target organs for vitamin D as well as an endocrine organ. Therefore, we include recent advances in this area including phosphorus and calcium handling by the kidney, localization of enzymes induced by 1,25$(OH)_2D_3$ along the nephron, that is, calcium binding protein and 24-hydroxylase, the receptors for 1,25$(OH)_2$D, and the proposed mechanisms of vitamin D action. Readers should refer to recent and, in our opinion, excellent review articles for broader aspects of vitamin D metabolism and action [1–4].

Localization of 1-hydroxylase

It has been well established that the site of 1-hydroxylase activity in the kidney is probably the proximal tubules [5, 6]. Indirect immunofluorescence studies show that cytochrome P-450-dependent mono-oxygenese, the enzyme thought to possess 1-hydroxylase activity, is localized predominantly in the proximal convoluted tubules as well as in the glomeruli [7]. Precise localization of 1-hydroxylase in the kidney was first presented by Brunette et al [8] using microdissected single nephron segments of vitamin D-deficient chick kidney: Significant enzyme activity was found only in the proximal convoluted tubules (PCT) and cortical thick loops, an extention of the PCT. In contrast to the relative ease of measuring 1-hydroxylase activity in vitro using various preparations of chick kidney, for example, homogenates, isolated mitochondria, dispersed tubule fragments, and so forth, it has been difficult to measure in vitro the enzyme activity of the mammalian kidney until recently perhaps because of inhibitors in the in vitro preparations of mammalian kidney [9]. After discovering that 1-hydroxylase activity could be measured in vitro in the homogenate of the fetal rabbit kidney [10], Akiba et al [11] demonstrated that 1-hydroxylase is localized in both PCT and the proximal straight tubules (PST) dissected from the fetal rabbit kidney. Using a similar preparation of dissected tubule segments, we demonstrated that the 1-hydroxylase is localized only in the PCT of vitamin D-deficient rat [12, 13]. It is of note that all the factors known to regulate 1-hydroxylase activity favor stimulation of the enzyme in vitamin D deficiency, for example, high parathyroid hormone (PTH) level, low endogenous 1,25$(OH)_2$D, low plasma concentrations of calcium and inorganic phosphate Pi ions [1–4]. Thus, one can assume that nephron segments other than the PCT do not possess any significant 1-hydroxylase activity in vitamin D deficient rats.

Peterson, Ghazarian, and Garancis [14] showed that 1-hydroxylase is localized in the nuclear preparation of the glomeruli and the mitochondria preparation of the proximal tubule fragments of vitamin D-deficient chick kidney. However, we with Torikai [13] as well as Akiba et al [11] and Brunette (personal communication) could not detect any significant enzyme activity in the glomeruli. The reasons for these seemingly discrepant results are not clear. It should be noted, however, that while the enzyme activities in the proximal tubules reported by Brunette et al [8], Akiba et al [11], and ourselves with Torikai [13] are comparable, the activity reported by Peterson, Ghazarian, and Garancis [14] are roughly two orders of magnitude lower as summarized in Table 1.

Localization of 24-hydroxylase in the kidney

In many ways the 24-hydroxylation reaction resembles 1-hydroxylation. Both require molecular oxygen, an atom of which is incorporated either in the 1- or 24-hydroxyl group [15]. Reduced NADP can be met equally by succinate, malate, or isocitrate oxidation by the mitochondria. Like 1-hydroxylase, addition of Pi (3 to 10 mM) tends to stimulate 24-hydroxylase whereas 1.0 mM Ca^{2+} is inhibitory [15]. Although isolation of 24-hydroxylase enzyme has, to our knowledge, not yet been reported, these properties of 24-hydroxylase suggest that it is probably a cytochrome P-450 enzyme like 1-hydroxylase. Based on these similarities of the two enzymes and the data that total P-450 concentration in the kidney does not change with vitamin D status in animals, Ghazarian [16] suggested the two distinct hydroxylation reactions might be catalyzed by a single enzyme system. Using an indirect immunofluorescence technique, he shows the predominant localization of ferredoxin, a

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Table 1. Localization and activity of 25(OH)D_3-1-hydroxylase along the nephron

Animals	Location of enzyme activity	Enzyme activity[a] *fmoles/mm/hr*	Enzyme activity[a] *fmoles/μg protein/hr*	References
Rat	PCT	0.65 ± 0.08	—	12, 41
	PST	0.69 ± 0.09	—	
Rabbit (fetus)	PCT	0.85 ± 0.42	3.67 ± 1.83	11
	PST	2.52 ± 1.03	12.5 ± 5.10	
Chick	PCT	1.76 ± 0.19	12.3 ± 1.4	8
	Cortical thick loop	1.73 ± 0.33	12.4 ± 2.4	
Chick	Glomerulus	—	0.12[b]	14
	PCT	—	0.17[b]	

Abbreviations: PCT, proximal convoluted tubules; PST, proximal straight tubules.

[a] Values are mean ± SEM.

[b] Values are expressed in [14] as 0.12 and 0.17 pmoles/mg protein/hr, respectively.

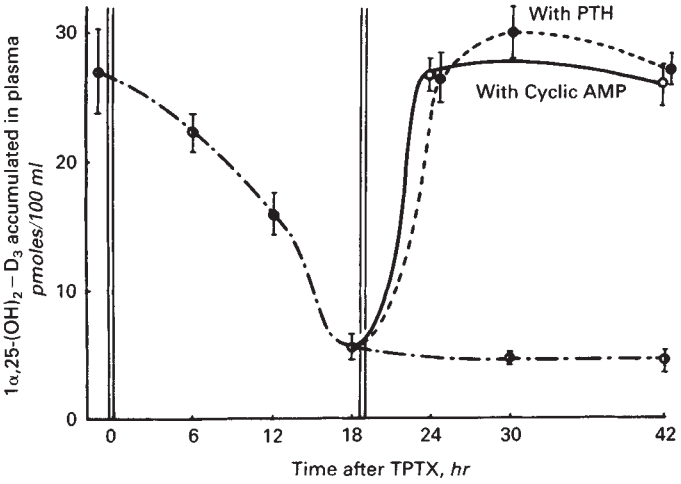

Fig. 1. *Effect and time course of TPTX and administration of PTH or cyclic AMP on the in vivo conversion of ^{3}H-25(OH)D_3 to ^{3}H-1,25(OH)$_2D_3$ in vitamin D-deficient rats.* The conversion rate was maximum at the doses of PTH and cyclic AMP given, 7.5 U/hr and 2 μ/mole/hr, respectively. (Reproduced from [21] with permission.)

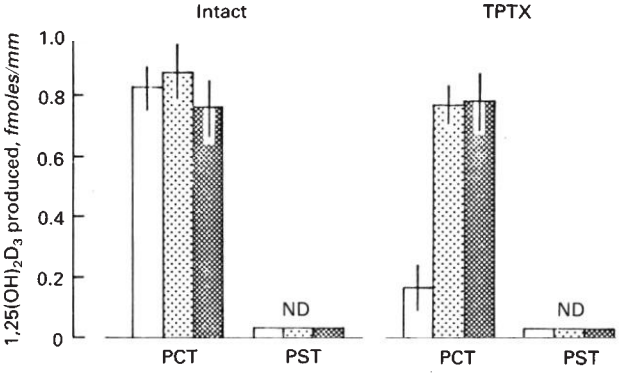

Fig. 2. *Effect of PTH and cyclic AMP on the 1-hydroxylase activity of the PCT and PST of intact and TPTX-vitamin D-deficient rats.* Twenty-four hours after sham or TPTX, rats were given 50 U 1–34 bovine PTH (*dotted columns*), 20 μ/mole cyclic AMP (*shaded columns*), or vehicle (*open columns*) every 2 hr s.c. Two hours after the fourth injection, rats were sacrificed and 1-hydroxylase was assayed in PCT and PST. Note that both PTH and cyclic AMP did not affect 1-hydroxylase activity in the PTH and cyclic AMP did not affect 1-hydroxylase activity in the PCT of vitamin D-deficient rats with intact parathyroid glands. In contrast, both PTH and cyclic AMP restored the reduced enzyme activity in the PCT of TPTX rats to the level of intact rats. Both PTH and cyclic AMP did not stimulate 1-hydroxylase in the PST. ND means not detectable. (Reproduced from [12] with permission.)

component of the P-450 enzyme system in proximal tubules and glomeruli [16].

To specifically define the localization of 24-hydroxylase, we measured the 24-hydroxylase activity in isolated single nephron segments. Our data show that the enzyme activity was detectable only in the PCT of vitamin D-replete rats and none in any other segments of vitamin D deficient or replete rats [13]. These data may seem to support Ghazarian's proposal that these two enzyme activities can be catalyzed by a single enzyme system. However, we have shown that with administration of 1,25(OH)$_2D_3$, 24-hydroxylase is induced not only in the PCT but also in the PST [13]. Moreover, recently Ichikawa, Hiwatashi, and Nishii [17] demonstrated the presence of an immunologically distinct enzyme for 1-hydroxylase, suggesting these two enzyme activities may be catalyzed by a different enzyme system.

Regulation of 1-hydroxylase

Parathyroid hormone (PTH). It has been established that PTH stimulates 1-hydroxylase activity and enhances renal production of 1,25(OH)$_2$D [12, 13, 18–21]. Several lines of evidence indicate that the secondary hyperparathyroidism plays an important role in 1-hydroxylase activation in vitamin D deficiency [13, 21]: Parathyrdoidectomy largely (~70 to 80%) abolishes the 1-hydroxylase activity in vitamin D deficiency and administration of PTH to thyroparapathyroidectomized (TPTX) vitamin D deficient rats restores the reduced enzyme activity to the level seen in the vitamin D deficient animal with intact parathyroid glands (Figs. 1 and 2). It is of interest that the maximum 1-hydroxylase activity obtained by PTH administration to parathyroidectomized (PTX) vitamin D deficient rats does not exceed the enzyme activity of vitamin D deficient rats with intact parathyroid glands. Moreover, exogenous PTH and cyclic AMP given to vitamin D deficient rats with intact parathyroid glands did not further increase the 1-hydroxylase in the PCT (Fig. 2). These observations support the notion that the 1-hydroxylase activity in the PCT of vitamin D deficiency is stimulated maximally by the secondary hyperparathyroidism.

The addition of PTH to kidney cells in vitro stimulates conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$ in a variety of preparations from several animal species [18, 22–28]. In the primary culture of chick kidney, stimulation of 1-hydroxylase by PTH requires the presence of insulin [29]; this stimulation needs several hours to appear both in vivo [30] and in vitro [31]. The in vivo effect is blocked by cycloheximide [30]. These results suggest that some new enzyme protein synthesis is involved in 1-hydroxylase activation by PTH. However, conflicting observations report the following: Rasmussen et al [18] demonstrated

that incubation of isolated chick tubules with the presence of PTH or cyclic AMP for 15 min caused a significant increase of $1,25(OH)_2D_3$ production. Larkins et al [22] also demonstrated similar effects of cyclic AMP in a similar chick tubule preparation, but this effect was not blocked by cycloheximide. Rost, Bikle, and Kaplan [28] demonstrated that PTH stimulates 1-hydroxylase in vitamin D-deficient chick kidney slices within 30 min. Thus, it seems that there may be two mechanisms of PTH dependent 1-hydroxylase stimulation: One is rapid appearing within minutes and *does not* need protein synthesis; another requires several hours and *does* involve new protein synthesis.

That the stimulatory effect of PTH is likely to be mediated by cyclic AMP is provided by the following observations: Cyclic AMP stimulates $1,25(OH)_2D_3$ production when added to isolated chick renal tubules and primary cell culture systems [22, 23, 27, 28]. Similar enzyme stimulation has been demonstrated in vivo by constant infusion of cyclic AMP into TPTX vitamin D-deficient rats [21]. The magnitude and time course of stimulation of $1,25(OH)_2D_3$ production by cyclic AMP were comparable to those of PTH [21]. Our recent data show that exogenous cyclic AMP restores the reduced 1-hydroxylase in PCT of TPTX vitamin D-deficient rats [32]. In addition, the effects of PTH and cyclic AMP are not additive [21], suggesting that both agents act on the same target cells and the effect of PTH is mediated by cyclic AMP. The presence of PTH-sensitive adenylate cyclase together with the 1-hydroxylase in the PCT of the rat kidney is consistent with the thesis.

Calcitonin. The effects of calcitonin on 1-hydroxylase have been controversial. Galante et al [33] first demonstrated that calcitonin injected subcutaneously every 6 hr for 48 hr stimulates the $1,25(OH)_2D_3$ production in vitamin-D deficient rats. Larkins, MacAuley, and MacIntyre [34] also demonstrated in vitro stimulation by calcitonin of 1-hydroxylase in isolated tubules from vitamin D-deficient chick kidney. By contrast, Lorenc et al [35] reported that the in vivo stimulation by calcitonin of $1,25(OH)_2D_3$ production in vitamin D-deficient rats was abolished by TPTX claiming that the stimulatory effect is secondary to an increase in PTH secretion due to a probable further decline in serum calcium. This is rather unlikely based on the results of Horiuchi et al [21] and ourselves [32] as already discussed, that is, that 1-hydroxylase is stimulated maximally by endogenous PTH in vitamin D deficiency and that injection of exogenous PTH or cyclic AMP in vitamin-D deficient rats with intact parathyroid glands failed to enhance $1,25(OH)_2D_3$ production. Furthermore, Horiuchi et al [36] demonstrated that calcitonin infused in vivo in TPTX vitamin D-deficient rats stimulated $1,25(OH)_2D_3$ production, an observation contrary to those of Lorenc et al [35].

The exclusive localization of the 1-hydroxylase in the PCT of mature vitamin D-deficient rats [13] is similar to that seen in the rachitic chick where the enzyme is localized in the PCT and cortical thick loops (considered to be an extension of PCT) [9]. However, our data obtained in the rat kidney are somewhat different from those of Akiba et al [11] who showed that both the PCT and PST are the sites of 1-hydroxylation in the fetal rabbit kidney. The lack of the enzyme activity in the PST of mature vitamin-D deficient rats but its presence in the fetal rabbit kidney may seem puzzling, possibly due to the species difference or the ontogeny of the enzyme activity. It is possible that the distribution of the 1-hydroxylase may change depending on the developmental stage of the animal (the ontogeny) and a greater demand for $1,25(OH)_2D_3$ may be met under certain circumstances, such as in the fetus, by recruiting a larger portion of the tubule, that is, PCT plus PST, producing $1,25(OH)_2D_3$. If this is the case, the factor(s) which regulates the ontogeny of 1-hydroxylase should be identified.

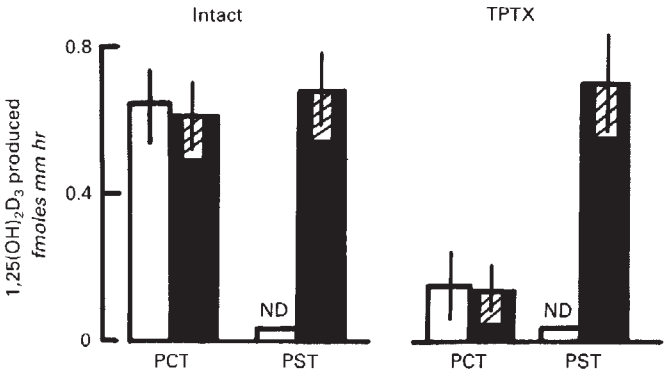

Fig. 3. *Effect of calcitonin on the 1-hydroxylase activity of the PCT and PST of vitamin D-deficient rats without (intact) or with TPTX.* Note that calcitonin stimulates 1-hydroxylase in the PST but not in the PCT in both intact and TPTX animals. *Open* and *closed bars* represent the enzyme activity in vehicle and calcitonin-treated rats, respectively. ND means not detectable. (Reproduced from [12] with permission.)

It has been shown that both PTH and calcitonin, when given in vivo, can stimulate the production of $1,25(OH)_2D_3$ from $25(OH)D_3$ in TPTX vitamin D-deficient rats [21]. Moreover, the in vivo effects of PTH and calcitonin are additive [36], a finding suggestive of different sites of action of these two hormones. In addition, plasma calcitonin levels are known to be elevated in the fetus of several mammalian species [37–40], while they are likely to be low in vitamin D-deficient rats due to hypocalcemia. These observations suggested to us that calcitonin could be the hormone-stimulating enzyme activity in the PST while PTH is primarily responsible for 1-hydroxylase activation. Such a postulate explains the presence of 1-hydroxylase in the PST of the fetal kidney but not in the PST of the mature vitamin D-deficient rat.

This hypothesis was tested by measuring 1-hydroxylase activity of nephron segments vitamin D-deficient rats given calcitonin [41]. As depicted in Fig. 3, the administration of calcitonin to vitamin D-deficient rats with or without TPTX stimulated 1-hydroxylase activity in the PST but did not affect the enzyme activity in the PCT. The enzyme activity remained undetectable in other nephron segments, including glomeruli, medullary and cortical thick ascending limbs of Henle, distal convoluted tubules, and collecting tubules. These data clearly showed that calcitonin selectively stimulates 1-hydroxylase in the PST and that calcitonin and PTH act on anatomically and functionally distinct segments of the nephron. These results explain the presence of 1-hydroxylase in the PST of the fetal kidney and its absence in the PST of the mature vitamin D-deficient rat. These data agree with in vivo observations that calcitonin stimulates the 1-hydroxylase activity independent of PTH and that the effects of PTH and calcitonin are additive [30, 36].

As mentioned above, PTH is believed to stimulate 1-hydroxylase via adenylate cyclase stimulation. By contrast, calcitonin

does not stimulate adenylate cyclase in the PST [41]. These data strongly suggest that calcitonin stimulates 1-hydroxylase in the PST via mechanisms independent of cyclic AMP. It is important to note that the administration of either PTH or cyclic AMP to vitamin D-deficient rats with or without TPTX did not stimulate 1-hydroxylase in the PST [32]. Thus, it is evident that the 1-hydroxylase in the PST is insensitive to cyclic AMP as well as to PTH.

To define the mechanism of calcitonin action, we have recently established a primary culture system of rat kidney and showed that calcitonin stimulates 1-hydroxylase activity when added to culture plates [25]. Similar results have been reported in the primary culture of mouse [42] and chick [22] kidney. These data indicate that calcitonin directly acts in the kidney. Matsumoto et al [43] recently reported that prostaglandin E_2 (PGE_2) antagonized the stimulatory effect of calcitonin on 1-hydroxylase when both agents are infused into TPTX vitamin D-deficient rats. However, in our primary culture system of rat kidney cells, we could not detect any effect of PGE_2 on the action of calcitonin, although PGE_2 did stimulate adenylate cyclase activity in this system, suggesting that the inhibitory effect of PGE_2 on the action of calcitonin might be indirect (unpublished data). Recent studies demonstrate that there are at least four intracellular messengers for action of peptide hormones, that is, cyclic AMP, cyclic GMP, calcium, and inositol-triphosphate. It is possible that calcium, inositol-triphosphate, or cyclic GMP might be involved in the action of calcitonin on the 1-hydroxylase in the PST. Recently, it has been reported that both human and salmon calcitonin stimulated cyclic GMP accumulation in human kidney cortical cells [44]. Further studies are needed to elucidate the mechanism of calcitonin in 1-hydroxylase activation.

The studies using microdissected single nephron segments revealed the presence of two distinct 1-hydroxylase systems in the kidney. One is localized in the PCT stimulated by PTH via cyclic AMP and thus activated in vitamin D deficiency: The other is in the PST, insensitive to PTH and cyclic AMP, thus latent in vitamin D deficiency, and stimulated by calcitonin via cyclic AMP-independent mechanisms (Fig. 4) [45, 46].

While the role of the PTH-sensitive 1-hydroxylase in the PCT in calcium metabolism has been well established, the biological significance of calcitonin-sensitive 1-hydroxylase may not be apparent. The thesis that the kidney is the sole organ endowed with the 1-hydroxylase has been based on data obtained in vitamin D-deficient animals in which the calcitonin-sensitive 1-hydroxylase is obviously latent. Thus, the discovery of 1-hydroxylase sensitive to calcitonin in the PST raises the possibility of the presence of 1-hydroxylase in tissues other than the kidney. Such 1-hydroxylase may be specifically responsive to calcitonin and latent in vitamin D deficiency. Recent evidence suggests $1,25(OH)_2D_3$ synthesis by the placenta and/or fetal tissues [47, 48], cultured bone cells [49, 50], and alveolar macrophages from patients with sarcoidosis [51]. It has yet to be shown whether these extrarenal 1-hydroxylases are sensitive to calcitonin.

The possible physiological significance of calcitonin sensitive 1-hydroxylase can be contemplated. In the fetus, serum ionized calcium concentrations are elevated, needing $1,25(OH)_2D_3$ to develop the skeleton. Were the fetus not endowed with the calcitonin-sensitive 1-hydroxylase, it might not be able to

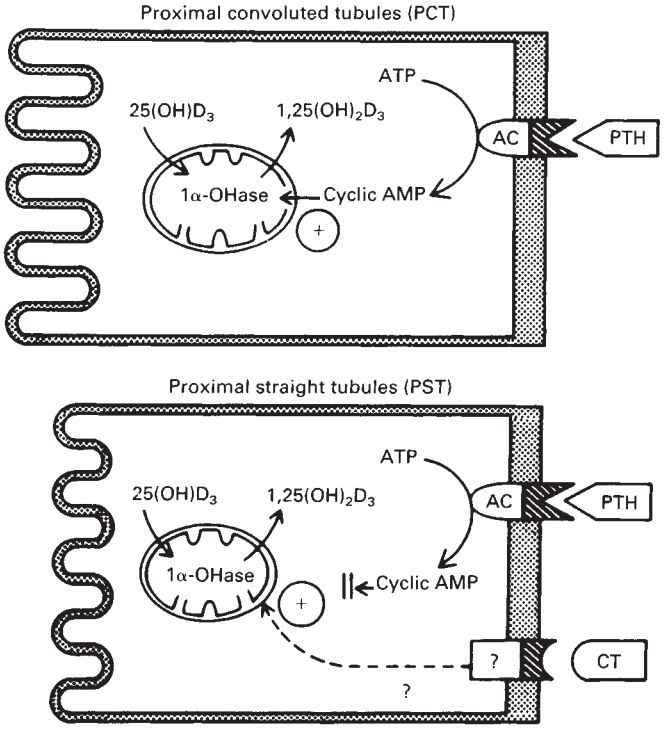

Fig. 4. *Model of two distinct 1-hydroxylase systems in the mammalian kidney.* (Reproduced from [12] with permission.)

recruit sufficient amounts of $1,25(OH)_2D_3$ because hypercalcemia may suppress PTH secretion while stimulating calcitonin secretion. Calcitonin is a hormone evolutionally older than PTH and one can postulate that the calcitonin-sensitive 1-hydroxylase may be important in a primitive life form or stage of life such as the fetus. Moreover, elevated plasma $1,25(OH)_2D_3$ levels during pregnancy [52] may be explained in part by high circulating calcitonin levels which may stimulate 1-hydroxylase in the placenta and the PST. These postulates, which have evolved from studies on nephron heterogeneity, certainly require further investigation.

Hydrogen ion concentration. Metabolic acidosis may alter vitamin D metabolism and may underlie metabolic bone diseases seen in patients with chronic metabolic acidosis. In both vitamin D-deficient rats [53] and chicks [54] with metabolic acidosis induced by ammonium chloride, in vivo conversion of ^3H-$25(OH)D_3$ to ^3H-$1,25(OH)_2D_3$ was suppressed. Also, the chick kidney 1-hydroxylase activity measured in vitro was diminished in acidosis [54, 55]. We also demonstrated that metabolic acidosis markedly suppressed the 1-hydroxylase in the PCT in vitamin D-deficient rats [56]. However, 16 hr of acidosis did not alter the enzyme activity [56].

In experiments with isolated renal tubules from the vitamin D-deficient chick, the reduction of pH to less than 7.0 reduced the enzyme activity [57]. In mitochondrial preparations from the chick kidney, the optimum pH for 1-hydroxylase activity is reported to be either 7.4 [58] or 6.9 [59]. We showed that the reduction in the pH of the incubation medium from 7.4 to 7.0 did not affect the enzyme activity in the PCT of vitamin D-deficient rats [56]. Lowering the perfusate pH from 7.4 to 7.1 does not affect 1-hydroxylase activity in the isolated perfused

rat kidney [60]. Thus, the suppression of the enzyme by acidosis does not seem to be due to a direct effect of low pH.

The mechanism responsible for suppression of 1-hydroxylase in metabolic acidosis remains unclear. As described above, PCT is the site of the elevated 1-hydroxylase in vitamin D-deficient rats, and this enzyme activity is regulated by PTH via cyclic AMP. Therefore, the suppression of 1-hydroxylase in the PCT of acidotic vitamin D-deficient rats could be due either to inhibition of PTH secretion or renal resistance to the action of PTH. Further, renal resistance to PTH action in the PCT could be at the step of PTH-dependent cyclic AMP generation or at steps beyond cyclic AMP production. A study from our laboratory showed that the excess PTH did not stimulate the suppressed 1-hydroxylase in the PCT of acidotic rats, but cyclic AMP fully restored the enzyme activity to normal [56]. These data strongly suggest that metabolic acidosis suppresses 1-hydroxylase activity in the PCT by inhibiting PTH-dependent adenylate cyclase activation. Moreover, the data indicate that metabolic acidosis does not affect the intracellular processes necessary for the 1-hydroxylase stimulation following cyclic AMP formation.

In addition, calcitonin stimulates the 1-hydroxylase activity of the PST of acidotic rats to levels similar to those observed in control rats treated with calcitonin [56]. These results show that the cyclic AMP-independent mechanisms in the PST stimulating 1-hydroxylase are not affected in metabolic acidosis.

Effects of vitamin D in the kidney

Phosphate handling. The vitamin D role in the renal handling of Pi has remained obscure due to conflicting results. Support for a stimulatory effect of vitamin D on renal Pi transport derives from clearance and micropuncture studies [61–64] describing the effects of vitamin D analogs in pharmacologic doses. Popovtzer et al [61], Pushchett, Moronz, and Kurnick [63] and Pushchett et al [62] have reported stimulation of Pi reabsorption by $1,25(OH)_2D_3$ which required the presence of PTH. Utilizing much lower doses of $1,25(OH)_2D_3$ in vitamin D-deficient TPTX rats small doses of PTH stimulate renal Pi reabsorption [65, 66]. Liang et al [67] and Bonjour, Preston, and Fleisch [68] have studied the effects of $1,25(OH)_2D_3$ on Pi uptake into renal cells isolated from rachitic chicks. Following in vivo administration of pharmacologic doses of $1,25(OH)_2D_3$, Pi uptake into tubule cells isolated increased after 3 hr, but it decreased in the cells isolated 17 hr after $1,25(OH)_2D_3$. They attributed the initial increase in Pi uptake to $1,25(OH)_2D_3$ and the later decrease in Pi uptake to an increase in the Pi load presented to the kidney in vivo [68]. They also showed $1,25(OH_2D_3$ added in vitro stimulates Pi uptake into renal tubule cells isolated from rachitic chicks [68]. Stimulation of Pi uptake was detectable with as little as 10^{-14} M $1,25(OH)_2D_3$, and was blocked by cycloheximide and actinomycin D. The effect of $1,25(OH)_2D_3$ peaked at 2 hr due to an increase in the V_{max} of Pi uptake. These results were most consistent with a genomic mechanism of action of $1,25(OH)_2D_3$ on Pi transport [68].

Inhibition of Pi transport by $1,25(OH)_2D_3$ has been reported in a series of studies [69–72]. The doses of $1,25(OH)_2D_3$ used in these studies were 120 pmoles iv once or 26 pmoles/day ip for 11 days, the doses required to normalize intestinal calcium and Pi absorption in TPTX rats [73], thus considered to be physiological. They assessed and compared Pi excretion during Pi infusion and Na-dependent Pi uptake into brushborder membrane vesicles (BBMV) isolated from the renal cortex [71]. Their data show that $1,25(OH)_2D_3$ decreases renal tubular Pi reabsorption and suggest that a functional role of $1,25(OH)_2D_3$ might maintain the tubular capacity to respond to a Pi load.

The reasons for these conflicting results on the effect of $1,25(OH)_2D_3$ on renal Pi transport are unclear. Several factors may have contributed. Some studies were performed in vitamin D-repleted animals in which the effects of vitamin D administration may differ from those in vitamin D-deficient animals. In addition, in studies utilizing vitamin D-deficient animals, rachitogenic diets, low in calcium and phosphorus content, produce hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism, all of which may affect tubular Pi handling independent of vitamin D [74]. Kurnik and Hruska [75] developed a normocalcemic, normophosphatemic model of mild vitamin D depletion in rats which shows little evidence of secondary hyperparathyroidism. Using this model, they examined the effects of vitamin D depletion and repletion with $1,25(OH)_2D_3$ on Pi transport in the brushborder membrane vesicle (BBMV). Partially vitamin D-deficient (PVDD) rats had increased Pi excretion, both absolute and fractional terms with a decrease in Na-dependent Pi uptake in BBMV. Administration of $1,25(OH)_2D_3$ to PVDD rats (15 pmoles/100 g body wt) 24 hr prior to the study decreased fractional excretion of Pi to the level of normal rats with an increase in Na-dependent Pi transport in BBMV. Larger doses of $1,25(OH)_2D_3$ produced hypercalcemia and hyperphosphatemia, an increase in Pi excretion, and a blunted response of Pi transport to $1,25(OH)_2D_3$. These latter data may partially explain the inhibitory effects of $1,25(OH)_2D_3$ reported in studies in which plasma Pi was not controlled and larger doses of $1,25(OH)_2D_3$ were used. These studies clearly demonstrated that vitamin D depletion is associated with decreased Pi reabsorption that is corrected rapidly by physiologic amounts of $1,25(OH)_2D_3$. The stimulatory effect of $1,25(OH)_2D_3$ on Pi transport is manifest at the level of the Na-dependent Pi transport in the brushborder membrane of proximal tubule cells.

Calcium handling. It has not been clear whether vitamin D, or one or more of its metabolites, exerts a direct effect on the renal tubular transport of calcium. Studies in humans as well as in experimental animals demonstrated that vitamin D has a hypocalciuric effect [76, 77], a calciuric effect [78, 79], or no effect at all [80–82]. These differing conclusions appear to arise from several reasons: the difference in the vitamin D status of the animals [77, 80], the difference in the doses and the kinds of vitamin D metabolites administered [77, 80], the changes in the serum calcium concentration and the filtered load of calcium [83], and the difference in the status of the function of PTH [78], a hormone that has a direct action on the renal tubular calcium reabsorption in the distal nephron [84–88]. Because one or more of these factors was not controlled or considered in many studies, it is often difficult to determine whether or not vitamin D has any direct action on the renal tubular transport of calcium.

In an attempt to critically examine this question, Yamamoto et al [88] have investigated the renal handling of calcium in rats in which the vitamin D status, the PTH status, and the serum calcium concentration were controlled independently. The apparent threshold of calcium excretion in vitamin D-deficient,

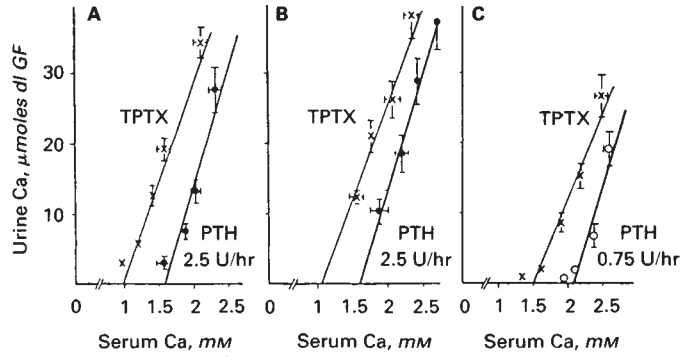

Fig. 5. *Effects of PTH infusion on the renal handling of calcium among three groups of TPTX rats.* Group A was fed a vitamin D-deficient normal calcium diet, group B a vitamin D-deficient high calcium diet, and group C a vitamin D-repleted diet. In groups A and B, PTH was given at 2.5 U/hr (●) and in group C at 0.75 U/hr (○). Renal calcium reabsorption is augmented or shifted to the right with PTH. However, a greater dose of PTH was required in vitamin D deficient rats (groups A and B) for a comparable shift in calcium reabsorption. Also, calcium threshold is lower in vitamin D deficiency with (group A) or without secondary hyperparathyroidism (group B) than in the vitamin D-repleted group (group C). (Reproduced from [88] with permission.)

TPTX rats was lower as compared with that in the vitamin D-repleted rat regardless of the presence of preceding hyperparathyroidism. PTH infused at 0.75 U/hr represents an estimated secretion rate of the hormone in vitamin D-repleted rat; the threshold of calcium excretion increased from 1.5 to 2.1 mM in vitamin D-repleted rats, while it was unchanged in vitamin D-deficient rats. A higher dose of PTH, 2.5 U/hr, an estimated secretion rate in vitamin D-deficient rat with secondary hyperparathyroidism, increased renal calcium threshold in vitamin D-deficient rats approximately by a similar magnitude from 1.0 to 1.6 mM (Fig. 5). The increase in the threshold in vitamin D-deficient rats was the same irrespective of the presence of preceding hyperparathyroidism. These data demonstrate that vitamin D deficiency decreases the effect of PTH to stimulate tubular calcium reabsorption both in the absence or presence of preceding hyperparathyroidism and that the vitamin D deficiency, per se, decreases tubular reabsorption of calcium [89]. Furthermore, they demonstrated that $1,25(OH)_2D_3$, but not $24,25(OH)_2D_3$, stimulates renal tubular calcium reabsorption (personal communication). These results clearly demonstrate evidence for a stimulatory effect of $1,25(OH)_2D_3$ on renal tubular calcium reabsorption.

Although the exact site of action of vitamin D on tubular calcium reabsorption remains to be determined, it is tempting to postulate that $1,25(OH)_2D_3$ may enhance the responsiveness of tubule to PTH at the cortical thick ascending loop of Henle and/or connecting tubules where the effect of PTH on tubular calcium transport has been reported [84–86, 88]

Localization of receptors for $1,25(OH)_2D_3$ (Table 2)

The mechanism of action of $1,25(OH)_2D_3$ involves binding of this hormone to cytoplasmic receptors and migration of the sterol-receptor complex to the nucleus leading to the induction of hormone-specific protein synthesis [1–4]. Recent studies demonstrated the presence of receptors for $1,25(OH)_2D_3$ in the kidney of mouse [90], rat [91], and chick [92], as well as the nuclear transfer of the $1,25(OH)_2D_3$-receptor complex in mouse

Table 2. Distribution of vitamin D metabolizing enzymes, vitamin D-dependent calcium binding protein and receptors for $1,25(OH)_2D_3$ along the rat nephron

Nephron segments	Enzymes	CaBP	Receptors	
			Autoradiography	Direct assay
Glm	+	−	+	Not tested
PCT	+	−	−	+
PST	+	−	−	Not tested
MTAL	−	−	+	+
CTAL	−	±	+	Not tested
DCT	−	+	+	Not tested
CNT	−	+	+	Not tested
CCT	−	±	−	Not tested
MCT	−	−	−	−
References	12, 14, 41, 104, 105	95–98	94	100

kidney [93]. It is also well established that at least two distinct proteins, that is, 24-hydroxylase and calcium-binding protein (CaBP), are induced by $1,25(OH)_2D_3$ in the kidney [1–4].

Recent autoradiographic data demonstrated that the nuclear uptake of $1,25(OH)_2[^3H]D_3$ in the kidney is localized predominantly in the distal nephron including medullary and cortical thick ascending limb of Henle (MTAL and CTAL), distal convoluted tubules (DCT), and, to some extent, in the glomerulus [94]. These data are consistent with findings of the exclusive localization of vitamin D-dependent CaBP in the distal nephron by immunohistochemical methods in the kidneys of mouse [95], rat [96–98], human [95, 96], rabbit [98], and chick [95, 98, 99]. Another protein induced by $1,25(OH)_2D_3$, 24-hydroxylase, has been localized exclusively in the proximal nephron [13]. Therefore, is seems reasonable that either receptor for $1,25(OH)_2D_3$ or another mechanism to recognize $1,25(OH)_2D_3$ must be present in the proximal nephron to transfer the hormonal signal to the site of protein synthesis. We examined this possibility using defined single nephron segments and demonstrated that the receptors for $1,25(OH)_2D_3$ are indeed localized both in proximal nephron (PCT) and distal nephron (MTAL) [100].

Vitamin D-dependent calcium binding protein. To our knowledge, Taylor and Wasserman [100] first demonstrated the presence of vitamin D-dependent CaBP in chick kidney. Christakos and Norman [101] reported that the vitamin D-dependent increase in chick kidney CaBP is accompanied by a parallel increase in the amount of mRNA coded for CaBP. Morrissey et al [94] were the first to localize CaBP in the kidney using immunohistological techniques with rabbit antibody against human renal CaBP. Their data showed that CaBP localizes in the distal tubule, collecting ducts, and the ascending loop of Henle in the human, dog, cat, rat, mouse, and chick kidney [95]. Christakos, Brunette, and Norman [98], using a radioimmunoassay with antisera prepared against vitamin D-dependent CaBP of the chick and microdissected single nephron segments of the chick kidney, showed that CaBP is localized in distal convoluted tubules (DCT), cortical collecting tubules, and thin loops of Henle. Rothen and Christakos [96] demonstrated, using rabbit antisera raised against chick CaBP, that CaBP is localized in DCT, the initial part of collecting tubules and in a lesser amount in the outer medullary collecting

duct in both human and rat kidneys. CaBP immunoreactive sites were found throughout the cytosol and the nuclear euchromatin. No preferential labeling of cellular membranes was found [96], suggesting CaBP may involve in regulating intracellular calcium, but not in the initial transmembrane transport of calcium [96]. Most recently, Taylor, McIntosh, and Bourdeau [97] reported using sheep anti-chick intestinal CaBP antisera that CaBP is localized in DCT, connecting tubules (CNT), and collecting tubules (the latter two have less amounts of CaBP) in rabbit kidney, DCT, CNT, and in a lesser amount in collecting tubules of rat kidney and exclusively in DCT in chick kidney [98].

Taken together, these data suggest that vitamin D-dependent CaBP is localized mostly in DCT, CNT, and a part of collecting tubules in all mammalian and chick kidneys, which agrees with the localization of receptors for $1,25(OH)_2D_3$ in these segments [94].

Induction of vitamin D metabolizing enzymes by $1,25(OH)_2D_3$ and its localization. Another protein known to be induced by $1,25(OH)_2D_3$ is the 24-hydroxylase. As described above, this enzyme is localized exclusively in the PCT of normal vitamin D-repleted rat kidneys. In contrast to the optimal in vivo condition for 1-hydroxylase activation in vitamin D deficiency, the lack of significant 24-hydroxylase activity does not necessarily rule out the presence of the enzyme in nephron segments other than the PCT. It is possible that enzyme activity may become manifest in other segments under stimulated conditions. Indeed, when 24-hydroxylase activity was measured in rats given $1,25(OH)_2D_3$, a most potent inducer of 24-hydroxylase [102, 103], the enzyme activity not only increased two- to threefold in the PCT but also became evident in the PST [13], with the specific activity of the enzyme becoming even higher in the PST than in the PCT [13]. No significant 24-hydroxylase activity was found in any other segments of the nephron even in $1,25(OH)_2D_3$-treated vitamin D-repleted rats [13]. It is reasonable to assume that this action of $1,25(OH)_2D_3$ to induce 24-hydroxylase is mediated by specific receptors; such $1,25(OH)_2D_3$ receptors should be localized in the proximal tubules. Indeed, as discussed above, we demonstrated the presence of the receptor for $1,25(OH)_2D_3$ in the PCT as well as in the MTAL [100].

Several other 25-hydroxyvitamin D metabolites have been reported to be produced by the kidney; this production may be stimulated by $1,25(OH)_2D_3$. While the receptors for $1,25(OH)_2D_3$ are also found in the CTAL and MTAL [100], proteins induced by $1,25(OH)_2D_3$ in these nephron segments have not been identified. It is possible, therefore, that one or more enzymes responsible for the production of other metabolites of vitamin D are localized in these nephron segments. To test these possibilities, we examined metabolism of $25(OH)_2D_3$ and $24,25(OH)_2[^3H]D_3$ using mitochondrial preparations and microdissected single nephron segments of rats treated with $1,25(OH)_2D_3$ [104, 105].

When mitochondria from $1,25(OH)_2D_3$-treated rat kidneys were incubated with $25(OH)D_3$, three major peaks were identified using LH-20 column chromatography. Two of them were identified as $24,25(OH)_2D_3$ and 24-oxo-$25(OH)D_3$, respectively, based on their chromatographic behaviors and chemical and spectroscopic features [104, 105]. Another peak has not yet been characterized fully, but it seems to be a lactone derivative based on its chromatographic behavior [105]

When the similarly prepared mitochondria were incubated with $24,25(OH)_2[^3H]D_3$, 24-oxo-$25(OH)D_3$, and the unidentified metabolite described above were also produced [104, 105]. To localize metabolite production sites, we first incubated mitochondria from renal cortex and medulla separately with either $25(OH)[^3H]D_3$. Cortical mitochondria produced all three metabolites, while medullary mitochondria failed to produce any of them, indicating that the enzymes are localized in the cortex. When metabolism of $1,25(OH)_2[^3H]D_3$ and $24,25(OH)_2$-$[^3H]D_3$ was examined using microdissected single nephron in the cortex, three metabolites are produced by both PCT and PST, but no significant amount of these metabolites was produced by any other nephron segments including glomeruli MTAL, CTAL, DCT, CNT, and CCT from either substrate [105]. These data are consistent with our earlier report that 24-hydroxylase is localized exclusively in the PCT and PST [13]. Furthermore, these data clearly demonstrate that 24-oxo-$25(OH)D_3$ and another unidentified metabolite are also produced in the PCT and PST. In addition, two minor metabolites were produced in both segments suggesting that enzymes responsible for the production of these additional minor metabolites may not be present in mitochondria but rather in other subcellular fractions such as microsome. Microsomal production of $25,26(OH)_2D_3$ has been reported [106]. The elution profile of $25,26(OH)_2D_3$ is similar to one of these two minor metabolites.

Although further characterization of these unidentified metabolites is needed, our data clearly demonstrate the localization of at least five different enzyme activities of vitamin D metabolism, including those responsible for the production of $24,25(OH)_2D_3$ and 24-oxo-$25(OH)_2D_3$ which are in the PCT and PST.

Mechanism of action of $1,25(OH)_2D_3$ in the kidney

It is widely recognized that steroid hormones act on target cells by a mechanism involving several specific steps: binding of the hormone to a cytosolic receptor, translocation of the hormone-receptor complex to the nucleus, and finally induction of protein synthesis. It is thought that $1,25(OH)_2D_3$ stimulates the synthesis of a specific CaBP in the intestine via this genomic mechanism [1–4]. $1,25(OH)_2D_3$ stimulates calcium reabsorption and also enhances the sensitivity to PTH to stimulate the tubular reabsorption of calcium [43]. Although the exact sites for this effect of $1,25(OH)_2D_3$ are unclear, it is tempting to propose that these sites are the CTAL [84–86], DCT [87], CNT [88], or all of these segments where PTH stimulates calcium transport. $1,25(OH)_2D_3$ alone can also stimulate tubular reabsorption of calcium in the kidney [43]. These actions of $1,25(OH)_2D_3$ seem to require new protein synthesis perhaps CaBP, since the response cannot be observed until several hours after the administration of $1,25(OH)_2D_3$, suggesting that this effect of vitamin D also involves genomic mechanism.

As mentioned earlier, $1,25(OH)_2D_3$ stimulates Pi uptake by the BBMV of proximal tubules which can be inhibited by actinomycin D or cycloheximide. These results, taken together with the presence of receptors for $1,25(OH)_2D_3$ in the proximal tubules, suggest $1,25(OH)_2D_3$ may affect Pi transport via a genomic mechanism. Also, the enzymes responsible for the

production of several metabolites of vitamin D are induced by $1,25(OH)_2D_3$ and localized in the proximal nephron [13, 104, 105] as described above; these effects may involve a genomic mechanism. It is unclear at present what a receptor role is for $1,25(OH)_2D_3$ in the MTAL because no protein has been identified which can be induced by $1,25(OH)_2D_3$ in this nephron segment.

Although a genomic mechanism is probably the major mechanism by which $1,25(OH)_2D_3$ exerts its effects in the kidney, nongenomic mechanisms may be involved in expression of some effects of $1,25(OH)_2D_3$. Edelman et al [107] reported that $1,25(OH)_2D_3$ elicited a change in cell membrane potential (V_m) within 1 min in proximal tubular cells of the necturus kidney whether it is injected in the lumen or peritubular capillaries. A similar quick action of $1,25(OH)_2D_3$ has been reported in the intestine: $1,25(OH)_2D_3$ increases the phosphatidylcholine content of the BBMV [108] and calcium uptake by BBMV within minutes [109]. These data clearly indicate that there is a nongenomic mechanism by which $1,25(OH)_2D_3$ affects transport function of these organs. Whether or not a receptor-mediated mechanism is involved in the changes of the V_m in the proximal tubules is unknown and needs further study.

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References

1. DeLuca HF, Schnoes HK: Metabolism and mechanism of action of vitamin D. *Ann Rev Biochem* 45:631–642, 1976
2. Norman AW: Vitamin D: The calcium homeostatic steroid hormone. Academic Press, New York, 1979
3. Fraser DR: Regulation of the metabolism of vitamin D. *Physiol Rev* 60:551–613, 1980
4. Haussler MR, McCain TA: Basic and clinical concepts related to vitamin D metabolism and action. *New Engl J Med* 297:874–983, 1041–1050, 1977
5. Midgett RJ, Spielvogel AM, Coburn JW, Norman AW: Studies on calciferol metabolism. VI. The renal production of the biologically active form of vitamin D, 1,25-dihydroxycholecalciferol; species, tissue and subcellular distribution. *J Clin Endocrinol Metab* 36:1153–1161, 1973
6. Sommerville BA, Fox J, Care AD: The in vitro metabolism of 25-hydroxycholecalciferol by pig kidney: effect of low dietary levels of calcium and phosphorus. *Br J Nutr* 40:159–162, 1978
7. Ghazarian JG, Garancis JC: Immunofluorescent localization of 25-hydroxyvitamin D_3-1α-hydroxylase ferredoxin in the renal tissue of chick. *J Histochem Cytochem* 27:1041–1045, 1979
8. Brunette MG, Chan M, Ferriere C, Roberts KD: Site of $1,25(OH)_2$-vitamin D_3 synthesis in the kidney. *Nature (London)* 276:287–289, 1978
9. Botham KM, Ghazarian JA, Kream BI, DeLuca HF: Isolation of an inhibitor of 25-hydroxyvitamin D_3-1-hydroxylase from rat serum. *Biochemistry* 15:2130–2135, 1976
10. Sunaga S, Horiuchi N, Takahashi N, Okuyama K, Suda T: The site of 1α,25-dihydroxyvitamin D_3 production in pregnancy. *Biochem Biophys Res Commun* 90:948–955, 1979
11. Akiba T, Endou H, Koseki C, Sakai F, Horiuchi N, Suda T: Localization of 25-hydroxyvitamin D_3-1α-hydroxylase in the mammalian kidney. *Biochem Biophys Res Commun* 94:313–318, 1980
12. Kawashima H, Kurokawa K: Unique hormonal regulation of vitamin D metabolism in the mammalian kidney. *Miner Electrolyte Metab* 9:227–235, 1983
13. Kawashima H, Torikai S, Kurokawa K: Localization of 25-hydroxyvitamin D_3-1α and -24-hydroxylase along the rat nephron. *Proc Natl Acad Sci USA* 28:1199–1203, 1981
14. Peterson EW, Ghazarian JG, Garancis JC: Mixed-function oxidase of 25-hydroxycholecalciferol in isolated chick kidney glomeruli: evidence for nuclear localization. *Calcif Tissue Int* 33:19–25, 1981
15. Madlhok TC, Schnoes HK, DeLuca HF: Mechanism of 25-hydroxyvitamin D_3-24-hydroxylation: incorporation of oxygen-18 into the 24 position of 25-hydroxyvitamin D_3. *Biochemistry* 16:2142–2145, 1977
16. Ghazarian JG: The 1α- and 24-hydroxylases of 25-hydroxycholecalciferol: one or two enzymes? in *Vitamin D Basic Research and Its Clinical Application,* edited by Norman AW, Schaefer K, Herrath DV, Grigoleit HG, Coburn JW, DeLuca HF, Mawer B, Suda T, Berlin, Walter de Gruyter 1979, pp 587–596
17. Ichikawa Y, Hiwatashi A, Nishii Y: Purification and reconstitution of vitamin D_3 25- and 1α-hydroxylase systems from bovine hepatic microsome and renal mitochondria, in *Vitamin D Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism,* edited by Normal AW, Schaefer K, Herrath DV, Grigoleit H-G, Berlin, Walter de Gruyter, 1982, pp 455–457
18. Rasmussen H, Wong M, Bikle D, Goodman DBP: Hormonal control of the renal conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. *J Clin Invest* 51:2502–2504, 1972
19. Garabedian M, Holick MF, DeLuca HF, Boyle IT: Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc Natl Acad Sci USA* 69:1673–1376, 1972
20. Fraser DR, Kodicek E: Regulation of 25-hydroxycholecalciferol-1α-hydroxylase activity in kidney by parathyroid hormone. *Nature New Biol* 241:163–166, 1973
21. Horiuchi N, Suda T, Takahashi H, Shimazawa E, Ogata E: In vivo evidence for the intermediary role of 3′, 5′-cyclic AMP in parathyroid hormone-induced stimulation of 1α,25-dihydroxyvitamin D_3 synthesis in rats. *Endocrinology* 101:969–974, 1977
22. Larkins RG, MacAuley SJ, Rapaport A, Martin TJ, Tulloch BR, Byfield PGH, Mattews EW, MacIntyre I: Effect of nucleotides, hormones, ions and 1,25-dihydroxycholecalciferol in 1,25-dihydroxycholecalciferol production in isolated chick renal tubules. *Clin Sci Mol Med* 46:569–583, 1974
23. Shain SA: The in vitro metabolism of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol by chick renal tubules. Effect of actinomycin D, puromycin, calcium, and parathyroid hormone. *J Biol Chem* 247:4404–4413, 1972
24. Fukase M, Birge SJ, Rifas L, Avichi LV, Chase LR: Regulation of 25-hydroxyvitamin D_3-1-hydroxylase in serum-free monolayer culture of mouse kidney. *Endocrinology* 110:1073–1075, 1982
25. Kawashima H, Iida-Klein A, Kurokawa K: Calcitonin stimulates 25-hydroxyvitamin D_3-1-hydroxylase in rat kidney cells in primary culture: A mechanism independent of cyclic AMP or prostaglandin synthesis (*abstract*). *Calcif Tissue Int* 36:517, 1984
26. Trechsel U, Bonjour J-P, Fleisch H: Regulation of the metabolism of 25-hydroxyvitamin D_3 in primary cultures of chick kidney cells. *J Clin Invest* 64:206–217, 1979
27. Bar A, Hurwitz S, Maoz A: The 25-hydroxycholecalciferol-1-hydroxylase activity of chick kidney cells: direct effect of parathyroid. *FEBS Lett* 113:328–330, 1980
28. Rost CR, Bikle D, Kaplan RA: In vitro stimulation of 25-hydroxycholecalciferol 1α-hydroxylation by parathyroid hormone in chick kidney slices: evidence for a role for adenosine 3′,5′-monophosphate. *Endocrinology* 108:1002–1006, 1981
29. Henry HL: Insulin permits parathyroid hormone stimulation of 1,25-dihydroxyvitamin D_3 production in cultured kidney cells. *Endocrinology* 108:733–735, 1981
30. Suda T, Horiuchi N, Akiba T, Sunaga S, Takahashi N: Localization and control mechanism for 25-hydroxyvitamin D_3-1α-hydroxylast activity in the mammalian kidney, in *Hormonal Control of Calcium Metabolism,* edited by Cohn DW, Talmage RV, Matthews JL, Amsterdam, Excerpta Medica, 1981, pp 212–221
31. Henry HL: Regulation of the hydroxylation of cell cultures of chick kidney cells. *J Biol Chem* 254:2722–2729, 1979
32. Kawashima H, Kurokawa K: Localization and hormonal reg-

ulation of 25(OH)D_3-1α- and 24-hydroxylases in the mammalian kidney, in *Vitamin D Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism,* edited by NORMAN AW, SCHAEFER K, HERRATH DV, GRIGOLEIT HG, Berlin, Walter de Gruyter, 1982, pp 449–454
33. GALANTE L, COLSTON KW, MACAULEY SJ, MACINTYRE I: Effect of calcitonin on vitamin D metabolism. *Nature (London)* 238:271–273, 1972
34. LARKINS RG, MACAULEY SJ, MACINTYRE I: Inhibitors of protein and RNA synthesis and 1,25-dihydroxycholecalciferol formation in vitro. *Mol Cell Endocrinol* 2:193–202, 1975
35. LORENC R, TANAKA Y, DELUCA HF, JONES G: Lack of effect of calcitonin on the regulation of vitamin D metabolism in the rats. *Endocrinology* 100:468–472, 1977
36. HORIUCHI N, SUDA T, TAKAHASHI H, SHIMAZAWA E, OGATA E: Salmon calcitonin-induced stimulation of 1α,25-dihydroxycholecalciferol synthesis in rats involving a mechanism independent of adenosine, 3′,5′-cyclic monophosphate. *Biochem J* 184:269–275, 1979
37. GAREL J-M, SAVAJOL H, BARLET J-P: Plasma immunoreactive calcitonin levels in pregnant ewes and their lambs. *Biol Neonate* 28:207–218, 1976
38. GAREL J-M, MARTIN-ROSSET W, BARLET J-P: Plasma immunoreactive calcitonin levels in pregnant mares and newborn foals. *Hormone Metabol Res* 7:429–432, 1975
39. REYNOLDS WA, PITKIN RM, WILLIAMS GA, BAUMAN AF, HARGIS GK, BELUHAN FZ, KAWAHARA W: Calcitonin responsiveness in the primate fetus. *J Clin Invest* 51:595–598, 1980
40. SAMMAN NA, ANDERSON GD, ADAM-MAYNE ME: Immunoreactive calcitonin in mother, neonate, child and adult. *Am J Obstet Gynec* 121:622–625, 1975
41. KAWASHIMA H, TORIKAI S, KUROKAWA K: Calcitonin selectively stimulates 25-hydroxyvitamin D_3-1α-hydroxylase in the proximal straight tubule of rat kidney. *Nature (London)* 291:327–329, 1981
42. FUKASE M, AVIOLI LV, BIRGE SJ, CHASE LR: Abnormal regulation of 25-hydroxyvitamin D_3-1α-hydroxylase activity by calcium and calcitonin in the renal cortex from hypophosphatemic (Hyp) mice. *Endocrinology* 114:1203–1207, 1984
43. YAMADA M, MATSUMOTO T, TAKAHASHI N, SUDA T, OGATA E: Inhibitory influence of prostaglandin E_2 on the renal effects of calcitonin in rats. *Abs 7th Int Congr Endocrinol,* July 1–7, Quebec City, Canada, 1984, pp 1383
44. ROTELLA CM, BRANDI ML, TOCCAFONDI RS: Human calcitonin increases both cyclic AMP and cyclic GMP accumulation in human kidney cells. *Eur J Pharmacol* 107:347–352, 1985
45. KUROKAWA K, KAWASHIMA H: Localization and hormonal regulation of 1,25$(OH)_2D_3$ in the kidney, in *Biochemistry of Kidney Functions,* INSERM Symposium No. 21, edited by MOREL F, Amsterdam, Elsevier Biomedical Press B.V., 1982, pp 329–336
46. WEISMAN Y, VARGAS A, DUCKETT G, REITER E, ROOT AW: Synthesis of 1,25-dihydroxyvitamin D_3 in the nephrectomized pregnant rat. *Endocrinology* 103:1992–1996, 1978
47. GRAY TK, LESTER GE, LORENC RS: Evidence for extrarenal 1α-hydroxylation of 25-hydroxyvitamin D_3 in pregnancy. *Science Wash* 204:1311–1313, 1979
48. TURNER RT, PUZAS EJ, FORTE MD, LESTER GE, GRAY TK, HOWARD GA, BAYLINK DJ: In vitro synthesis of 1α,25-dihydroxycholecalciferol and 24,25-dihydroxycholecalciferol by isolated calvarial cells. *Proc Natl Acad Sci USA* 77:5472–5724, 1980
49. HOWARD GA, TURNER RT, SHERRARD DJ, BAYLINK DJ: Human bone cells in culture metabolize 25$(OH)D_3$ to 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$. *J Biol Chem* 256:7738–7740, 1981
50. ADAMS JS, SHARMA OP, GACAD MA, SINGER FR: Metabolism of 25-hydroxyvitamin D_3 by cultured pulmonary alveolar macrophages in sarcoidosis. *J Clin Invest* 72:1856–1860, 1983
51. KUMAR R, COHEN WR, SILVA P, EPSTEIN FH: Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J Clin Invest* 63:342–344, 1979
52. LEE SW, RUSSEL J, AVIOLI LV: 25-Hydroxycholecalciferol to 1,25-dihydroxycholecalciferol: conversion impaired by systemic metabolic acidosis. *Science* 195:994–996, 1977
53. SAUVEUR B, GARABEDIAN M, FELLOT C, MOUGIN P, BALSAN S: The effect of induced metabolic acidosis on vitamin D_3 metabolism in rachitic chicks. *Calcif Tissue Res* 23:121–124, 1977
54. CUNNINGHAM J, BIKLE DD, FAUSTO A, AVIOLI LV: Effect of acidosis on renal 25-hydroxyvitamin D_3-1α- and 24-hydroxylase (*abstract*). *Calcif Tissue Int* 33:333, 1981
55. KAWASHIMA H, KRAUT JA, KUROKAWA K: Metabolic acidosis suppresses 25-hydroxyvitamin D_3-1α-hydroxylase in the rat kidney: distinct site and mechanism of action. *J Clin Invest* 70:135–140, 1982
56. BIKLE DD, RASMUSSEN H: The ionic control of 1,25-dihydroxyvitamin D_3 production in isolated chick renal tubules. *J Clin Invest* 55:292–298, 1975
57. GRAY RW, OMDAHL JL, GHAZARIAN JG, DELUCA HF: 25-Hydroxycholecalciferol-1α-hydroxylase. Subcellular location and properties. *J Biol Chem* 247:7528–7532, 1972
58. BIKLE DD, RASMUSSEN H: The ionic control of 1,25-dihydroxyvitamin D_3 synthesis in isolated chick renal mitochondria. The role of calcium as influenced by inorganic phosphate and hydrogen ion. *J Clin Invest* 55:229–304, 1975
59. REDDY GS, JONES G, FRAZER DR: Effects of acute metabolic acidosis on the conversion of 25$(OH)D_3$ to 1,25$(OH)_2D_3$ by the isolated perfused rat kidney, in *Hormonal Control of Calcium Metabolism,* edited by COHN DV, TALMAGE RV, METHEWS JL, Amsterdam, *Excerpta Medica,* 1981, pp 381
60. GEKLE D, STRODER J, ROSTOCK D: The effect of vitamin D on renal inorganic phosphate reabsorption of normal rats, parathyroidectomized rats, and rats with rickets. *Pediatr Res* 5:40–52, 1971
61. POPOVTZER MM, ROBINETTE JB, DELUCA HF, HOLICK MF: The acute effect of 25-hydroxycholecalciferol on renal handling of phosphrus. *J Clin Invest* 53:913–921, 1974
62. PUSHCHETT JB, FERNANDEZ PC, BOYLE IT, GRAY RW, OMDAHL JL, DELUCA HF: The acute renal tubular effects of 1,25-dihydroxycholecalciferol. *Proc Soc Exp Biol Med* 141:379–384, 1972
63. PUSHCHETT JB, MORONZ J, KURNICK WS: Evidence of a direct action of cholecalciferol and 25-hydroxy cholecalciferol on the renal transport of phosphate, sodium, and calcium. *J Clin Invest* 51:373–385, 1972
64. EGEL J, PFANSTIEL J, PUSCHETT JB: Effects of 1,25-dihydroxyvitamin D_3 on brush border vesicle transport (*abstract*). *Clin Res* 24:28A, 1983
65. GEORGAKI H, PUSCHETT J: Acute effects of a physiological dose of 1,25-dihydroxy vitamin D_3 on renal phosphate transport. *Endocrinol Res Commun* 9:135–143, 1982
66. LIANG CT, BARNES J, BALAKIR R, CHENG L, SACKTOR B: In vitro stimulation of phosphate uptake in isolated chick renal cells by 1,25-dihydroxycholecalciferol. *Proc Natl Acad Sci* 79:3532–3536, 1982
67. LIANG CT, BARNES J, CHENG L, BALAKIR R, SACKTOR B: Effects of 1,25$(OH)_2D_3$ administered in vivo on phosphate uptake by isolated chick renal cells. *Am J Physiol* 242:C312–C318, 1982
68. BONJOUR JP, PRESTON C, FLEISCH H: Effect of 1,25-dihydroxy vitamin D_3 on the renal handling of P_i in the thyroparathyroidectomized rats. *J Clin Invest* 60:1419–1428, 1977
69. MUHLBAUER RC, BONJOUR JP, FLEISCH H: Tubular handling of P_i localization of effects of 1,25$(OH)_2D_3$ and dietary P_i in TPTX rats. *Am J Physiol* 241:F123–F128, 1981
70. STOLL R, KINNE R, MURER H, FLEISCH H, BONJOUR JP: Phosphate transport by rat renal brush border membrane vesicles: influence of dietary phosphate, thyroparathyroidectomy, and 1,25-dihydroxy vitamin D_3. *Pflügers Arch* 380:47–52, 1979
71. STOLL R, KINNE R, MURER H: Effect of dietary phosphate intake on phosphate transport by isolated rat renal brush border vesicles. *Biochem J* 180:465–470, 1979
72. RIZZOLI R, FLEISCH H, BONJOUR P: Role of 1,25-dihydroxy vitamin D_3 on intestinal phosphate absorption in rats with a normal vitamin D supply. *J Clin Invest* 60:639–647, 1977
73. AMIEL C, KUNTZIGER H, COUETTE S, COUREAU C, BERGONIOUX N: Evidence for a parathyroid-hormone-independent calcium modulation of phosphate transport along the nephron. *J Clin Invest* 57:256–263, 1976
74. KURNIK BR, HRUSKA KA: Effects of 1,25-dihydroxycholecalci-

ferol on phosphate transport in vitamin D-deprived rats. *Am J Physiol* 247:F177–F182, 1984
75. PUSCHETT JB, MORANZ J, KURNICK WS: Evidence for a direct action of cholecalciferol and 25-hydroxycholecalciferol on the renal transport of phosphate, sodium, and calcium. *J Clin Invest* 51:373–385, 1972
76. COSTANZO LS, SHEEHE PR, WEINER IM: Renal action of vitamin D in D-deficient rats. *Am J Physiol* 226:1490–1495, 1974
77. BONJOUR J-P, TRECHSEL U, FLEISCH H, SEHENK R, DELUCA HF, BAXTER LA: Action of 1,25-$(OH)_2$-D_3 and a diphosphonate on calcium metabolism in rats. *Am J Physiol* 229:489–495, 1975
78. RIZZOLI R, FLEISCH H, BONJOUR J-P: Effect of thyroparathyroidectomy on calcium metabolism in rats: role of $1,25(OH)_2D_3$. *Am J Physiol* 233:E160–E164, 1977
79. HUGI K, BONJOUR J-P, FLEISCH H: Renal handling of calcium: influence of parathyroid hormone and 1,25-dihydroxyvitamin D_3. *Am J Physiol* 236:F349–F356, 1979
80. BERNSTEIN D, KLEEMAN CR, MAXWELL MH: The effect of calcium influsions, parathyroid hormone, and vitamin D on renal clearance of calcium. *Proc Soc Exp Biol Med* 112:353–355, 1963
81. BURNATOWSKA MA, HARRIS CA, SUTTON RAL, SEELY JF: Effects of $1,25(OH)_2D_3$ on Ca, Mg and Pi excretion in the hamster *(abstract)*. *Kidney Int* 12:454, 1977
82. STEEL TH, ENGLE JE, TANAKA Y, LORENC RS, DUDGEON KL, DELUCA HF: Phosphatemic action of 1,25-dihydroxyvitamin D_3. *Am J Physiol* 229:489–495, 1975
83. SUKI WN, ROUSE D: Hormonal regulation of calcium transport in thick ascending limb renal tubules. *Am J Physiol* 241:F171–F174, 1981
84. BOURDEAU JE, BURG MB: Effect of PTH on calcium transport across the cortical thick ascending limb of Henle's loop. *Am J Physiol* 239:F121–F126, 1980
85. IMAI M: Effects of parathyroid hormone and N^6,O^2-dibutyryl cyclic AMP on Ca transport across the rabbit distal nephron segments perfused in vitro. *Pflügers Arch* 390:145–151, 1981
86. SHAREGHI GR, STONER LC: Calcium transport across segments of the rabbit distal nephron in vitro. *Am J Physiol* 235:F367–F375, 1978
87. IMAI M: The connecting tubule: A funtional subdivision of the rabbit distal nephron segments. *Kidney Int* 15:346–356, 1979
88. YAMAMOTO M, KAWANOBE Y, TAKAHASHI H, SHIMAZAWA E, KIMURA S, OGATA E: Vitamin D deficiency and renal calcium transport in the rat. *J Clin Invest* 74:507–513, 1984
89. COLSTON KW, FELDMAN D: Demonstration of a 1,25-dihydroxycholecalciferol cytoplasmic receptor-like binder in mouse kidney. *J Clin Endocrinol Metab* 49:798–800, 1979
90. CHANDLER JS, PIKE JW, HOUSSLER MR: 1,25-dihydroxyvitamin D_3 receptors in rat kidney cytosol. *Biochem Biophys Res Commun* 90:1057–1063, 1979
91. CHRISTAKOS S, NORMAN AW: Studies on the mode of action of calciferol XVIII: Evidence for a specific high affinity binding protein for 1,25-dihydroxyvitamin D_3 in chick kidney and pancreas. *Biochem Biophys Res Commun* 89:56–63, 1979
92. COLSTON K, FELDMAN D: Nuclear translocation of the 1,25-dihydroxycholecalciferol receptor in mouse kidney. *J Biol Chem* 255:7510–7513, 1980
93. STUMPF WE, SAR M, NARBAITZ R, REID FA, DELUCA HF, TANAKA Y: Cellular and subcellular localization of $1,25-(OH)_2D_3$ in rat kidney. Comparison with that of parathyroid hormone and estradiol. *Proc Natl Acad Sci USA* 77:1149–1153, 1980
94. MORRISSEY RL, BUCCI TJ, EMPSON RN JR, LUFKIN EG: Calcium-binding protein: its cellular localization in jejunum, kidney and pancreas. *Proc Soc Exp Biol Med* 149:56–60, 1975
95. BOTH J, BROWN D, NORMAN AW, ORCI L: Localization of vitamin D-dependent calcium binding protein in mammalian kidney. *Am J Physiol* 243:F243–252, 1982
96. RHOTEN WB, CHRISTAKOS S: Immunocytochemical localization of vitamin D-dependent calcium binding protein in mammalian nephron. *Endocrinology* 109:981–983, 1981
97. TAYLOR AN, MCINTOSH JE, BOURDEAU JE: Immunocytochemical localization of vitamin D-dependent calcium binding protein in renal tubules of rabbit, rat and chick. *Kidney Int* 21:765–773, 1982
98. CHRISTAKOS S, BRUNETTE MG, NORMAN AW: Localization of immunoreactive vitamin D-dependent calcium binding protein in chick nephron. *Endocrinology* 109:322–324, 1981
99. KAWASHIMA H, KUROKAWA K: Localization of 1,25-dihydroxyvitamin D_3 receptors along the rat nephron: evidence for the presence of specific binding in both proximal and distal nephron. *J Biol Chem* 257:13428–13432, 1982
100. TAYLOR AN, WASSERMAN RH: Vitamin D_3-induced calcium-binding protein: partial purification, electrophoretic visualization and tissue distribution. *Arch Biochem Biophys* 119:536–540, 1967
101. CHRISAKOS S, NORMAN AW: Studies on the mode of action of calciferol XXIX. Biochemical characterization of 1,25-dihydroxyvitamin D_3 receptors in chick pancreas and kidney cytosol. *Endocrinology* 108:140–149, 1981
102. TANAKA Y, LORENC RS, DELUCA HF: The role of 1,25-dihydroxyvitamin D_3 and parathyroid hormone in the regulation of chick renal 25-hydroxyvitamin D_3 and parathyroid hormone in the regulation of chick renal 25-hydroxy-vitamin D_3-24-hydroxylase. *Archs Biochem Biophys* 171:521–526, 1975
103. HENRY HL: Regulation of the hydroxylation of 25-hydroxyvitamin D_3 in vivo and in primary cultures of chick kidney cells. *J Biol Chem* 254:2722–2729, 1979
104. KAWASHIMA H, IIDA-KLEIN A, KUROKAWA K: Unidentified metabolites of vitamin D_3 produced in the kidney: Induction by 1,25-dihydroxyvitamin D_3 and site of production, in *Endocrine Control of Bone and Calcium Metabolism,* edited by COHN DV, FUJITA T, POTTS JT JR, TALMAGE RV, Amsterdam, *Excerpt Medica,* vol. 8A, 1984, pp 429
105. KAWASHIMA H, IIDA-KLEIN A, KUROKAWA K: Sites of action of 1,25-dihydroxyvitamin D_3 in the kidney, in *Endocrine Control of Bone and Calcium Metabolism,* COHN CV, FUJITA T, POTTS JT JR, TALMAGE RV, Amsterdam, Elsevier Science Publishers, B.V., 1984, vol. 8B, pp 231–232
106. THIERRY-PALMER M, GRAY TK, WILLIAMS ME, TALMAGE RV: Rat kidney microsomes convert 25-hydroxyvitamin D_3 to an unidentified metabolite. *Biochem Biophys Res Commun* 110:766–771, 1983
107. EDELMAN A, THIL CL, GARABEDIAN M, ANAGNOSTOPOULOS T, BALSAM S: Genome independent effects of 1,25-dihydroxy vitamin D_3 on membrane potential. *Biochim Biophys Acta* 732:300–303, 1983
108. MATSUMOTO T, FONTAINE O, FASMUSSEN HJ: Effect of $1,25(OH)_2D_3$ on phospholipid metabolism in chick duodenal mucosal cell. *J Biol Chem* 256:3354–3360, 1981
109. RASMUSSEN H, FONTAINE O, MAX EE, GOODMAN DBP: The effect of lα-hydroxyvitamin D_3 administration on calcium transport in chick intestine brush border membrane vesicles. *J Biol Chem* 254:2993–2999, 1979